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NOTES

Autoradiographic Localization of [^{3}H]Gentamicin in the Proximal Renal Tubules of Mice

MICHAEL J. KUHAR,[1] LINDA L. MAK,[2] AND PAUL S. LIETMAN[1, 2]*
Departments of Pharmacology and Experimental Therapeutics[1] and Medicine,[2] The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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The site of localization of [^{3}H]gentamicin within mouse kidney is shown to be the proximal renal tubule by coincidence of the radioactivity, as visualized by autoradiography, and the mucopolysaccharide-rich microvilli characteristic of proximal convoluted tubules, as visualized by histochemical staining.

The extensive accumulation and avid retention of aminoglycoside antibiotics by the kidneys of both experimental animals (4, 9, 13) and humans (1, 3) may be related to the nephrotoxicity associated with these drugs. If these processes are related, then an understanding of the precise anatomic localization of aminoglycoside accumulation and retention may be fundamental to the prevention and treatment of aminoglycoside-induced nephrotoxicity. We have investigated the intrarenal localization of the aminoglycoside gentamicin in the mouse kidney.

Three male mice (40- to 55-g Swiss mice; Buckberg, Tomkins Cove, N.Y.) were injected with 10 mg (5 ml/kg) of [^{3}H]gentamicin per kg (0.9 mCi/mg, Amersham Corp.; diluted to 50 μCi/mg with gentamicin sulfate from Sigma) in 0.2% ethanol. Three control male mice were injected with 5 ml of 0.2% ethanol per kg, and both kidneys from each of these mice were prepared and examined in the same manner as for the treated mice. Twenty-four hours later, both kidneys from each mouse were removed under pentobarbital anesthesia, and sections of the medial parts of the kidneys (both axes were sampled in different kidneys) were mounted on a copper microtome chuck and frozen in liquid nitrogen slush. Four-micrometer sections of these tissues were either thaw-mounted or dry-mounted onto emulsion-coated (Kodak NTB-3) slides and stored for approximately 1 month. This autoradiographic procedure has been developed to prevent or minimize the diffusion of small molecules from tissue sites (11). After development of the emulsion, the tissues were stained either with pyronine-y or by the periodic acid-Schiff technique, which visualizes mucopolysaccharide-rich microvilli characteristic of cells in the proximal convoluted tubules.

A representative photograph of the resultant autoradiograph and histochemical staining is shown in Fig. 1. All kidney preparations from treated animals showed similar findings. Dense clusters of autoradiographic grains (ARG) were restricted to cortical regions of the kidney, where they were associated with most, but not all, tubule profiles. The ARG were strikingly absent over glomeruli and over structures in the medulla including the thin limbs, collecting ducts, and blood vessels. A very slight enrichment of ARG was observed over the straight parts of the tubules below the cortical layers containing the glomeruli. In periodic acid-stained sections, the ARG were found exclusively over tubules that stained positively for mucopolysaccharides near the lumen. Furthermore, the convoluted tubules with the dense cluster of ARG had larger cells when compared with the tubules with no ARG. Control slides prepared for positive and negative chemography showed no spurious generation of or fading of latent images. The same localization has also been visualized in rat kidney preparations (data not shown).

From these data it is concluded that [^{3}H]gentamicin is very selectively localized to the proximal convoluted tubules. This is based on the observations that the ARG are strikingly localized to the kidney cortex where the proximal tubules are found, and only the tubules with ARG had periodic acid-Schiff-positive borders and large epithelial cells—both features being characteristic of proximal rather than distal convoluted tubules. Since the ARG varied over the tubule profiles, it seems that either different proximal tubules retained the drug in different amounts or different parts of the tubules differ in their ability to take up and retain the drug.

Aminoglycoside antibiotics have been previ-

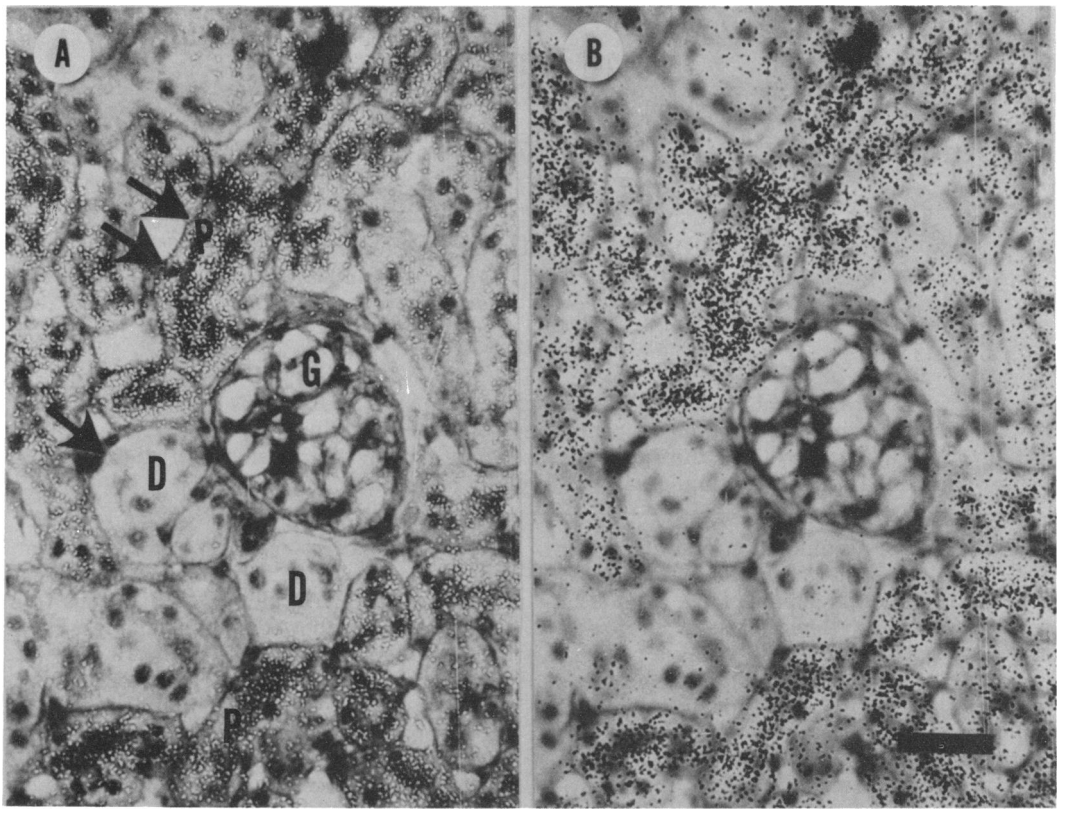

FIG. 1. *Mouse kidney cortex with tissue in focus (A) and ARG in focus (B). A glomerulus (G), two representative distal tubules (D), and two representative proximal tubules (P) are shown. The glomeruli as well as many tubules (single arrow) had no overlying grains. Other tubules with periodic acid-Schiff-positive cells (double arrow) had a high density of overlying ARG. Slide was exposed for 28 days. Bar = 30 μm.*

ously shown to accumulate in the kidneys, achieving concentrations considerably higher than those in the plasma or most other organs (1, 3, 4, 9, 13). Within the kidney, most of the aminoglycoside is found in the cortex as compared with the medulla (4, 9). This renal accumulation is a result of the prolonged retention of aminoglycosides by the kidney as compared with the plasma or other tissues (4, 9).

We have provided evidence that, within the renal cortex, the aminoglycoside is confined to the proximal renal tubule. Just et al. have reported the localization of gentamicin to the tubules in the cortical areas of the kidneys of mice (7). Our data confirm and extend these observations by matching the gentamicin-containing tubules with those that stain for mucopolysaccharides (proximal tubules) and by demonstrating that this localization persists for at least 24 h. Also, our observations have been made on unfixed and quickly frozen sections as opposed to fixed specimens, thus avoiding the possibility of an artifact introduced by fixing the specimens. Our results also illustrate the lack of aminoglycoside in glomeruli and distal tubules extending into the cortex.

It is interesting that the localization of the aminoglycoside coincides with the site of histological damage to the kidneys as described by others (2, 5, 6, 8, 10, 12, 14). Thus, it seems likely that the accumulation and retention of aminoglycosides by the proximal renal tubules is related to the morphological damage to those tubules.

The mechanism responsible for this localized accumulation of aminoglycosides by the proximal renal tubule remains to be defined. From evidence derived by autoradiography (7; R. Jerrauld and F. J. Silverblatt, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 17th, New York, N.Y., Abstr. no 349, 1977) and by studies in perfused rat kidneys (V. U. Collier, W. E. Mitch, and P. S. Lietman, Clin. Res. **26:** 288A, 1978) the entrance into the proximal renal tubule, appears to be primarily from the luminal side. The mechanism of cellular toxicity subse-

quent to accumulation also remains to be defined.

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